signals from 2 protons in HYSCORE measurements. These results suggest the possibility that an additional residue contributes to the stability of Q_B^-. Supported by NSF grant MCB 08-18121.

2912-Pos

Investigations of Q_A Binding Pocket Mutations in Rhodobacter Sphaeroides Reaction Centers Using Eseem and Hyscore

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The photosynthetic reaction center of *Rhodobacter sphaeroides* contains two identical ubiquinone molecules. The redox potentials of these two quinones are significantly different, and are tuned by the specific interactions with the protein environment of the binding pocket. The primary quinone (Q_A) has hydrogen bonds between the two carbonyls - O_4 to the N_δH of His M219 and O_1 to the peptide NH of Ala M260. Small changes in this hydrogen bond structure could induce large changes to redox potential, as suggested by the effect of mutating the isoleucine at M265 to threonine, which induces a 100 mV change in the E_m of Q_A. In order to better understand the nature of this redox tuning, M265 mutants have been analyzed with x-band ESEEM and HYSCORE. These techniques are very sensitive to the coupling of the semiquinone anion to surrounding nitrogen nuclei. The results show significant changes to the coupling between Q_A and nitrogens from both M260 and M219. Peaks associated with histidine N_δ show a substantially different NQI asymmetry parameter (η) than the wildtype. However, results from ^{15}N labeled samples show that hyperfine coupling is fairly similar. This could suggest a rotation of the NQI tensor versus the g-tensor of the semiquinone, which could, in turn, indicate a large change of local electric field at the N_δ. Supported by NSF grant MCB 08-18121.

2913-Pos

Structural Design, Assembly and Engineering of Oxidoreductases Engaged in Energy Conversion

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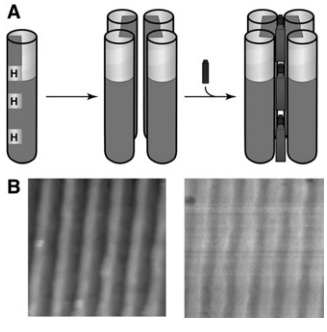
Many key cell functions are accomplished through complicated system of enzymes and redox carrier molecules that control electron and proton transport. Although significant number of these enzymes has been structurally characterized, the actual mechanism of redox catalysis is not always understood. Therefore we have adopted a different approach to address the structure-function relationship of oxidoreductases: we aim to uncover the assembly instructions required for function using smaller, simpler, more robust model proteins, maquettes. Our questions ask how many engineering elements are required to achieve a particular biological function, what are the individual biochemical and structural tolerances of these elements and how much of a protein infrastructure is consumed in accommodating the function. To start answering these questions, we have synthesized a set of amphiphilic maquettes. Our tetrameric maquettes assemble with up to six ferric hemes B per tetramer in three different positions (Figure A). These maquettes transfer electrons across membranes, bind O2 and CO and assemble into single monolayers on electrodic surfaces including planar gold and graphite. Using nanolithography and scanning probe microscopy, we measured topology (Figure B left), impedance, potential (Figure B right) and current on the nanoscale level.



2914-Pos

Initial Characterization of a New Class of 2Fe-2S Proteins from the Plant Arabidopsis Thaliana

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A new class of 2Fe-2S proteins has been identified with 3Cys and 1His coordination (1,2). In an attempt to investigate the function of these NEET proteins, we have initiated studies in *Arabidopsis thaliana* that contains a single copy of a similar gene called At5g51720. The transcript levels of At5g51720 were shown to increase in response to stress (3). Here we report the isolation and initial characterization of At5g51720 protein. Its Vis absorbance spectrum is nearly identical with the human homolog and is similarly reversibly reducible (Figure). The 2Fe-2S clusters are also similarly labile. Thus, the At5g51720 protein serves as a good model for investigations of the physiological function of the NEET protein family.

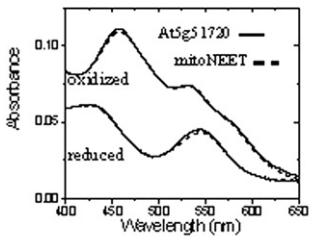

Figure. Oxidized (upper) and reduced (lower) spectra of *Arabidopsis* At5g51720 and Human mitoNEET.

(1) Paddock *et al.* (2007) *Proc Natl. Acad. Sci USA* **104**, 14342-14347.
(2) Conlan *et al.* (2009) *J. Mol. Biol.* **392,** 143-153.
(3) Camp *et al.* (2004) *Plant Cell* **15,** 2320-2332.

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2915-Pos

Time-Resolved Thermodynamics of Inter-Molecular Electron Transfer Between Water-Soluble Anionic Free-Base Porphyrins and Ubiquinone

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Inter-molecular electron transfer (ET) between ubiquinone (ubiQ) and biological molecules represents a critical process in cellular respiration. The biological importance of this process has stimulated research efforts to understand the fundamental mechanisms associated with proton-coupled ET involving ubiQ. In the present study the kinetics and time resolved thermodynamics associated with photoinduced intermolecular ET between the singlet states of two anionic porphyrins, meso-tetrakis(4-carboxyphenyl)porphyrin (4CP) and meso-tetrakis(4-sulfonatophenyl)porphyrin (4SP), and ubiQ are reproted. The anionic porphyrins facilitate charge separation between the porphyrin cation radical and the ubiQ anion radical. Addition of ubiQ to solutions of either 4SP or 4CP result in quenching of the porphyrin excited singlet state, τ(4SP/4CP)~10-11ns and τ(4SP/4CP-ubiQ)~8ns, with the quenching rate constants, k_q, of $5x10^{10}$ $M^{-1}s^{-1}$ and $2x10^{10}$ $M^{-1}s^{-1}$ respectively. Time-resolved photoacoustic calorimetry (PAC) signals for both porphyrin species in the presence of ubiQ are monophasic (τ<50ns) with respect to a calorimetric reference at pH 9 and 7 and is independent of ubiQ concentration up to 1.4μM (initial concentration of 4SP/4CP ~6-10μM). At pH 7 and 9 ΔV is independent of ubiQ concentration with a volume contraction of ~2-3 mL mol^{-1} and expansion of ~20 mL mol^{-1} respectively. Biphasic signals are observed for each species at pH 6: a fast phase <50ns and slow phase ~300-400ns. The observed ΔH for the fast phase are independent of concentration within experimental error while those for the slow phase are reported as a function of ubiQ concentration.

2916-Pos

Structural and Electronic Insights into Tryptophan Radicals in Proteins

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Tryptophan radicals play significant roles in mediating biological electron transfer reactions and catalytic processes. Despite their prevalence in Nature, there is a dearth of knowledge on the structure and dynamics of these transient species. Here, we report absorption, EPR, and resonance Raman spectra of long-lived solvent-exposed and buried tryptophan neutral radicals in azurin mutants. These spectra reveal important markers and trends that reflect the local environment, structure, hydrogen bonding state, and protonation state of the radical; comparison of these spectra to those of the closed-shell counterparts indicates that the spectral features of the radical are highly sensitive to structure and environment. The results and analysis described here not only provide detailed basis spectra for ongoing work, but also shed light on the nature of proton-coupled electron transfer reactions.

2917-Pos

Microbial Nanowire Electronic Structure Probed by Scanning Tunneling Microscopy

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We have studied the pilus nanowires expressed by the bacterium, *Geobacter sulfurreducens*, using high resolution scanning tunneling microscopy (STM). *G. sulfurreducens* is a metal reducing bacterium that has evolved electrically conductive pili to efficiently transfer electrons across large distances. Here we employ the electronic sensitivity of STM to resolve the molecular substructure and the local density of states along the nanowire, in an effort to elucidate the mechanism of conduction.